CLAIMS

What is claimed is:

Sub B1

1. Purified hCOMP prepared by the method comprising:

a) introducing DNA encoding hCOMP into cells, thereby producing cells expressing hCOMP;

b) culturing the cells in a culture medium under conditions suitable for expressing the hCOMP, thereby producing expressed hCOMP; and

c) purifying the hCOMP in the presence of calcium.

2. The purified hCOMP of claim 1 wherein the hCOMP is purified under calcium-replete conditions.

3. The purified hCOMP of claim 1 wherein the cells in step b) are cultured in a calcium-replete culture medium.

4. The purified hCOMP of claim 2 wherein the calcium is present at millimolar levels when the hCOMP is purified.

5. The purified hCOMP of claim 1 wherein the hCOMP is purified in a solution characterized by a calcium concentration of at least 300 *u*M.

6. The purified hCOMP of claim 1 wherein the hCOMP is expressed and purified in a solution characterized by a calcium concentration of at least 300 *u*M.

7. The purified hCOMP of claim 1 wherein the cells expressing hCOMP are produced by introducing into cells DNA encoding full length hCOMP.

8. A method for producing purified hCOMP comprising:

a) obtaining DNA encoding full length hCOMP;

b) introducing the DNA into cells, thereby producing cells expressing hCOMP;

c) culturing the cells in a culture medium under conditions suitable for expressing the hCOMP, thereby producing expressed hCOMP; and

d) purifying the hCOMP in the presence of calcium.

9. The method of claim 8 wherein the hCOMP is purified under calcium-replete conditions.

10. The method of claim 8 wherein the hCOMP is expressed and purified in a solution characterized by a calcium concentration in the millimolar range.

11. The method of claim 8 wherein the hCOMP is expressed and purified in a solution characterized by a calcium concentration of at least 300 *u*M.

12. Purified hCOMP which digests into bands of ~~50 kDa~~ or 55 kDa when cleaved by trypsin.

13. Purified hCOMP which digests into bands of 62 kDa or 67 kDa when cleaved by ~~trypsin.~~

14. An antibody to the hCOMP of claim 1.

15. An antibody to hCOMP, wherein the hCOMP is purified by the method of claim 8.

16. The antibody of claim 14 which is monoclonal or polyclonal.

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17. An ELISA kit comprising the hCOMP of claim 1.

18. An ELISA kit comprising at least one antibody to the hCOMP of claim 1.

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19. An ELISA kit comprising hCOMP produced by the method of claim 8.

20. An ELISA kit comprising at least one antibody to the hCOMP produced by the method of claim 8.

21. An implant comprising a matrix comprising human cartilage oligomeric matrix protein (hCOMP) of claim 1, wherein the matrix comprises at least one material selected from the group consisting of: treated cartilage and bone matrices, collagens, hyaluronan, fibrin gels, carbon fibers, porous polylactic acid, type I collagen gel and type II collagen gel.

22. The implant of claim 21 wherein said matrix is seeded with cells selected from the group consisting of: mesenchymal stem cells or chondrocytes.

23. The implant of claim 21 wherein a differentiation agent is bound to the hCOMP, wherein the differentiation agent is vitamin D_3 at least one vitamin D_3 metabolite, or retinoic acid.

24. The implant of claim 21 further comprising at least one proteoglycan.

25. The implant of claim 24 wherein the proteoglycan is chondroitin sulfate proteoglycan.

26. An implant comprising a matrix comprising human cartilage oligomeric matrix protein (hCOMP) of claim 1 bound to a differentiation agent, wherein the matrix comprises at least one material selected from the group consisting of: treated cartilage and bone matrices, collagens, hyaluronan, fibrin gels, carbon fibers, porous polylactic acid, type I collagen gel and type II collagen gel.

27. The implant of claim 26 further comprising chondroitin sulfate proteoglycans.

28. The implant of claim 26 wherein the matrix comprises type I collagen gel or type II collagen gel.

29. The implant of claim 26, wherein said matrix is seeded with chondrogenic cells.

30. The implant of claim 29 wherein the chondrogenic cells are mesenchymal stem cells or chondrocytes which are embedded in the matrix.

31. The implant of claim 26 wherein the differentiation agent is vitamin D_3, a vitamin D_3 metabolite or retinoic acid.

32. The implant of claim 31 wherein the vitamin D_3 metabolite is selected from the group consisting of 1,25-dihydroxyvitamin D_3 and 24R,25-dihydroxyvitamin D_3.

33. The implant of claim 26 wherein the hCOMP is recombinant human COMP.

34. The implant of claim 26 wherein the hCOMP is purified human COMP secreted by cells cultured in a calcium-replete environment and purified in the presence of calcium.

35. The implant of claim 26 wherein the implant further comprises at least one proteoglycan.

36. The implant of claim 35 wherein the proteoglycan is a chondroitin sulfate proteoglycan.

37. A composition comprising purified COMP and a biological matrix, wherein the matrix comprises at least one material selected from the group consisting of: treated cartilage and bone matrices, collagens, hyaluronan, fibrin gels, carbon fibers, porous polylactic acid, type I collagen gel and type II collagen gel and purified COMP.

38. The composition of claim 37 further comprising chondrocytes or mesenchymal stem cells.

39. The composition of claim 37 wherein the COMP is bound to a differentiation agent.

40. The composition of claim 37 further comprising chondroitin sulfate proteoglycans.

41. The composition of claim 37 wherein the COMP is hCOMP purified in a calcium-replete environment.

42. The composition of claim 37 wherein the biological matrix comprises type I collagen gel or type II collagen gel.

43. A method of repairing cartilage at a cartilage defect area comprising implanting into the defect area a matrix comprising hCOMP of claim 1 bound to a differentiation agent.

44. The method of claim 43 wherein the matrix further comprises type 1 collagen gel or type II collagen gel.

45. The method of claim 43 wherein chondroitin sulfate proteoglycans are added to the matrix prior to implantation.

46. The method of claim 43 wherein the matrix is seeded with chondrogenic cells selected from the group consisting of: mesenchymal stem cells and chondrocytes.

47. The method of claim 43 wherein the differentiation agent is vitamin D_3 or vitamin D_3 metabolites or retinoic acid.

48. The method of claim 47 wherein at least one vitamin D_3 metabolite is selected from the group consisting of: 1,25-dihydroxyvitamin D_3 and 24R,25-dihydroxyvitamin D_3.

49. The method of claim 43 wherein the hCOMP is recombinant.

50. The method of claim 43 wherein the hCOMP is secreted by cells cultured in a calcium-replete environment and purified in the presence of calcium.

51. The method of claim 43 wherein the matrix further comprises at least one proteoglycan and wherein the hCOMP acts as a bridge between the collagen and the proteoglycan.

52. The method of claim 51 wherein the proteoglycan is a chondroitin sulfate proteoglycan.

53. A method for repairing cartilage comprising applying an amount of a composition to a site in need of cartilage repair, said composition comprising a matrix, wherein the matrix comprises at least one material selected from the group consisting of: treated cartilage and bone matrices, collagens, hyaluronan, fibrin gels, carbon fibers, porous polylactic acid, type I collagen gel, type II collagen gel and purified hCOMP.

54. The method of claim 53 wherein said composition further comprises chondrocytes or mesenchymal stem cells.

55. The method of claim 53 wherein the hCOMP is bound to a differentiation agent.

56. The method of claim 53 wherein the composition further comprises chondroitin sulfate proteoglycans.

57. A method of producing cartilage at a cartilage defect area comprising implanting into the defect area a matrix comprising hCOMP of claim 1 bound to a differentiation agent, wherein the matrix includes at least one material selected from the group consisting of: treated cartilage and bone matrices, collagens, hyaluronan, fibrin gels, carbon fibers, porous polylactic acid, type I collagen gel and type II collagen gel.

58. A method for making an implant for cartilage repair comprising:

a) binding a differentiation agent to hCOMP, thereby forming differentiation agent-bound hCOMP; and

b) adding the differentiation agent-bound hCOMP to a matrix, wherein the matrix includes at least one material selected from the group consisting of: treated cartilage and bone matrices, collagens, hyaluronan, fibrin gels, carbon fibers, porous polylactic acid, type I collagen gel and type II collagen gel,

whereby the hCOMP mediates delivery of the differentiation agent to chondrocytes and release of the differentiation agent, and serves as a chemoattractant for chondrocytes.

59. A method for making an implant for cartilage repair comprising:

a) binding a differentiation agent to hCOMP, thereby forming differentiation agent-bound hCOMP;

b) adding the differentiation agent-bound hCOMP to a matrix which includes at least one material selected from the group consisting of: treated cartilage and bone matrices, collagens, hyaluronan, fibrin gels, carbon fibers, porous polylactic acid, type I collagen gel and type II collagen gel; and

c) adding chondrogenic cells to the matrix,

whereby the hCOMP mediates delivery to the cells of the differentiation agent and release of the differentiation agent, helping to maintain and promote the chondrogenic cells to mature and differentiate, thereby producing naturally occurring non-traumatic cartilage.

60. The method of claim 59 wherein the differentiation agent is vitamin D_3 or vitamin D_3 metabolites or retinoic acid.

61. The method of claim 59 wherein the matrix further comprises growth factors.

62. A method of transplanting autologous chondrocytes comprising culturing chondrocytes isolated from a patient on tissue culture plates coated with hCOMP purified in the presence of calcium, said COMP bound with a differentiation agent thereby creating expanded chondrocytes, whereby the hCOMP mediates attachment of the expanded chondrocytes and provides delivery and release of the differentiation agent.

63. The method of claim 62 wherein the differentiation agent is vitamin D_3 or vitamin D_3 metabolites or retinoic acid.

64. The method of claim 62 comprising the additional steps of injecting the expanded chondrocytes in the presence of hCOMP bound with a differentiation agent into the defect area, thereby aiding in the maintenance of differentiated chondrocytes and stimulating production of type II collagen and other cartilage components by the chondrocytes in the defect areas.

65. The method of claim 62 wherein the hCOMP is produced by the method of claim 8.

66. A method of transplanting autologous chondrocytes comprising injecting chondrocytes in the presence of hCOMP, alone or bound with a differentiation agent, into a defect area, thereby aiding in maintenance of differentiated chondrocytes and stimulating production of type II collagen and other cartilage components by the chondrocytes in the defect areas.

67. The method of claim 66 wherein the hCOMP is produced by the method of claim 8.

68. A method of producing chondrocytes for autologous transplantation comprising culturing chondrocytes isolated from a patient on tissue culture plates coated with COMP bound with a differentiation agent, thereby creating expanded chondrocytes, whereby the COMP mediates attachment of the expanded chondrocytes and provides a differentiation agent.

69. Chondrocytes produced by the method of Claim 53.

70. A method of mediating attachment of chondrocytes in autologous transplantation comprising injecting the chondrocytes in the presence of differentiation agent-bound COMP into the defect area, thereby creating and aiding in the maintenance of the differentiation stage of chondrocytes and stimulating production of type II collagen and other cartilage components by the chondrocytes in the defect areas.

71. A method of preparing a cartilage repair implant comprising culturing cells expressing COMP, purifying COMP in a calcium-replete environment and adding it to a matrix comprising at least one material selected from the group consisting of: treated cartilage and bone matrices, collagens, hyaluronan, fibrin gels, carbon fibers, porous polylactic acid, type I collagen gel and type II collagen gel.

72. The method of claim 71 further comprising seeding the matrix with chondrocytes or mesenchymal stem cells prior to implantation.

73. A method of making an implant for cartilage repair comprising:

a) culturing chondrogenic cells in vitro in the presence of purified hCOMP bound to a differentiation agent; and

b) seeding said chondrogenic cells into a matrix comprising at least one material selected from the group consisting of: treated cartilage and bone matrices, collagens, hyaluronan, fibrin gels, carbon fibers, porous polylactic acid, type I collagen gel and type II collagen gel.

74. The method of claim 73 wherein the cells of step b) are seeded into the matrix in the presence of COMP.

75. The method of claim 73 wherein the COMP is expressed by chondrocytes, tendon or ligament cells, smooth muscle cells, pericytes, or human embryonic kidney cells transfected with full length COMP.

76. An assay for determining the amount of COMP in a biological sample comprising:

a) incubating the biological sample with an anti-COMP antibody produced against COMP purified in the presence of calcium, under conditions suitable for binding the antibody to COMP in the biological sample, thereby producing a solution of bound antibody and unbound antibody;

b) adding the solution of a) to a plate and incubating the plate under conditions suitable for binding the unbound antibody of a) to COMP, wherein the plate has been coated with purified COMP, thereby producing antibody bound to the plate;

c) after washing the plate, detecting antibody bound to the plate; and

d) comparing the detected antibody of step c) with detected antibody of a similarly processed control in which known amounts of COMP are

tested instead of the biological sample, thereby determining the amount of COMP in the biological sample.

77. An assay for determining the amount of hCOMP in a biological sample comprising:

a) incubating the biological sample with the antibody of claim 15 under conditions suitable for binding the antibody to hCOMP in the biological sample, thereby producing a solution of bound antibody and unbound antibody;

b) adding the solution of a) to a plate and incubating the plate under conditions suitable for binding the unbound antibody of a) to hCOMP, wherein the plate has been coated with purified hCOMP, thereby producing antibody bound to the plate;

c) after washing the plate, detecting antibody bound to the plate; and

d) comparing the detected antibody of step c) with detected antibody of a similarly processed control in which known amounts of hCOMP are tested instead of the biological sample, thereby determining the amount of hCOMP in the biological sample.

78. The assay of claim 77 wherein the biological sample is serially diluted human sera or synovial fluid.

79. The assay of claim 77 wherein the anti-COMP antibodies are detected with an enzyme conjugated secondary antibody.

80. An assay to detect anti-COMP antibodies in a biological sample comprising:

a) coating the hCOMP of claim 1 on a plate;

b) serially diluting the sample, thereby creating serial dilutions;

c) contacting the serial dilutions with the plate; and

d) detecting the presence of bound anti-hCOMP antibodies with an enzyme-conjugated antibody.

81. The assay of claim 77 wherein the biological sample is selected from the group consisting of serum and synovial fluid.

82. A method of diagnosing the presence or progression of arthritis in a mammal comprising detecting and measuring COMP by the assay of claim 76 and comparing the amount of COMP measured with the amount of COMP in a control mammal that does not have arthritis.

83. The method of claim 82 wherein the arthritis is rheumatoid arthritis or osteoarthritis.

84. The method of claim 77 wherein the anti-COMP antibodies are polyclonal antibodies or monoclonal antibodies.

85. A method of detecting degradation of COMP comprising detecting COMP with an immunoblot assay using anti-COMP antibodies of claim 15 that recognize the degraded form of COMP and not the non-degraded form of COMP.

86. The method of claim 85 wherein the anti-COMP antibodies are polyclonal antibodies or monoclonal antibodies.

87. An assay for determining the amount of degraded COMP in a biological sample comprising:

a) incubating the biological sample with antibodies recognizing degraded COMP but not non-degraded COMP under conditions suitable for binding the antibodies to degraded COMP in the biological sample, thereby producing a solution of bound antibodies and unbound antibodies;

b) adding the solution of a) to a plate and incubating the plate under conditions suitable for binding the unbound antibodies of a) to degraded COMP, wherein the plate has been coated with degraded COMP, thereby producing antibodies bound to the plate;

c) after washing the plate, detecting the antibodies bound to the plate; and

d) comparing the detected antibodies of step c) with a similarly processed control in which known amounts of degraded COMP are tested instead of the biological sample, thereby determining the amount of degraded COMP in the biological sample.

88. A method of diagnosing inflammatory joint disease in a mammal comprising detecting degradation of COMP in synovial fluid by the method of Claim 87 and comparing the amount of degraded COMP measured to the amount of degraded COMP in a mammal which does not have inflammatory joint disease.

89. The method of claim 88 wherein the inflammatory joint disease is rheumatoid arthritis or osteoarthritis.